RESEARCH

Relationships of Resistance to Fusarium Ear Rot and Fumonisin Contamination with Agronomic Performance of Maize

Leilani A. Robertson-Hoyt, Craig E. Kleinschmidt, Don G. White, Gary A. Payne, Chris M. Maragos, and James B. Holland*

ABSTRACT

Resistance to Fusarium ear rot [caused by *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F. moniliforme* Sheldon) (teleomorph: *Gibberella moniliformis*) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*)] and fumonisin contamination is heritable and controlled by at least 11 gene regions in a maize (*Zea mays* L.) population created by backcrossing the highly resistant donor line, GE440, to the susceptible but commercially successful recurrent parent line, FR1064. The relationship between resistances to Fusarium ear rot and fumonisin contamination and agronomic performance has not been reported. Therefore, the objective of this study was to examine the relationship between disease resistance and agronomic utility in this population by measuring resistances to Fusarium ear rot and fumonisin contamination in $BC_1F_{1:2}$ lines, and yield and agronomic performance in topcrosses of these lines. Fumonisin contamination was not correlated with yield, but two fumonisin quantitative trait loci (QTL) mapped to similar positions as yield QTL. Fusarium ear rot had a small positive correlation with topcross yield ($r = 0.29$), but QTL for the two traits mapped to distinct genomic positions. Similar results for other traits indicate that QTL can contribute in opposite directions to the overall genetic correlations between traits and that some trait correlations arise in the absence of detectable QTL effects on both traits. In general, no strong relationships were observed between disease resistance traits and agronomic traits, thus selection for increased resistance should not unduly affect agronomic performance.

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Abbreviations: BIC, Bayesian Information Criterion; CIM, composite interval mapping; MIM, multiple interval mapping; PCR, polymerase chain reaction; QTL, quantitative trait locus (loci); SSR, simple sequence repeat.

Fusarium verticillioides (Sacc.) Nirenberg (synonym *F. moniliforme* Sheldon) (teleomorph: *Gibberella moniliformis*) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*) cause Fusarium ear rot of maize (*Zea mays* L.). These fungi can also produce fumonisins, a family of mycotoxins that can harm animals and humans that consume fumonisin-contaminated grain (Munkvold and Desjardins, 1997). These toxins appear to cause a number of human and animal diseases (Colvin and Harrison, 1992; Gelderblom et al., 1988; Hendricks, 1999; Rheeder et al., 1992; Ross et al., 1992). In response, the United States Food and Drug Administration has published a *Guidance for Industry* that suggests limiting fumonisin concentrations to between 2 and 4 $\mu g\ g^{-1}$ (2 and 4 ppm) for milled corn products used for human consumption (CFSAN, 2001a, 2001b).

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To limit the negative impacts of fumonisin contamination and Fusarium ear rot, selection for resistance to these two aspects of the disease is an important objective. Robertson et al. (2006) reported high family mean heritabilities and high positive genotypic correlations between fumonisin contamination and Fusarium ear rot, suggesting that phenotypic selection should be effective at improving resistance to these traits. The relationship between these disease resistance traits and other important agronomic traits also impacts the feasibility of developing cultivars with improved resistance, especially if the sources of disease resistance are unadapted exotic germplasm. Negative genetic relationships between disease resistance and other agronomic traits may occur due to negative pleiotropy or to repulsion-phase linkages between favorable alleles at disease resistance loci and at genes affecting agronomic traits. Conversely, positive genetic relationships between disease resistance and yield may occur due to the protection afforded by resistance genes against the yield-reducing effects of ear rot.

Robertson et al. (2006) studied the inheritance of resistance to Fusarium ear rot and fumonisin contamination in a population derived from the first backcross of GE440 to FR1064. GE440 has high levels of resistance to both Fusarium ear rot and fumonisin contamination (Robertson et al., 2006) but is an older inbred line developed from the Hastings Prolific open-pollinated variety (Jenkins and Robert, 1959) that is not acceptable for commercial hybrid production. The earliest published record of GE440 indicates that it was used in field studies in 1951 (Jenkins et al., 1957). In contrast, FR1064 is a widely used B73-type commercial inbred developed by Illinois Foundation Seeds (Clements et al., 2004). Therefore, further backcrossing of resistance alleles from GE440 into the FR1064 background via phenotypic selection and marker-assisted selection will be conducted with this population. However, the question remains regarding what the effects of selection for increased disease resistance on other agronomic traits will be in this backcrossing program.

The relationship between resistances to Fusarium ear rot and fumonisin contamination and agronomic performance has not been previously reported. The objectives of this study were to investigate the relationships between disease resistance traits measured in partially inbred lines and agronomically important traits (grain yield, grain moisture, lodging, plant and ear height, and silk and anthesis dates) measured in topcrosses of these lines to a common tester. Relationships among traits were studied with correlation analysis and comparison of quantitative trait loci (QTL) mapped for the different traits on a common linkage map. These two analyses provided complementary approaches to study the genetic relationship between Fusarium ear rot and fumonisin contamination resistance and other phenotypic traits.

MATERIALS AND METHODS

Population Development

GE440 was identified in preliminary studies as a potential source for resistance to Fusarium ear and kernel rot and low fumonisin contamination (Clements et al., 2004; Robertson et al., 2006; Robertson-Hoyt et al., 2006). A segregating population derived from the cross FR1064 × GE440 was created. Resistant inbred GE440 was crossed and backcrossed once to the susceptible inbred FR1064, and BC_1F_1 plants were self-pollinated to form 213 $BC_1F_{1:2}$ families. Topcross hybrids of FR1064, GE440, and of the 213 $BC_1F_{1:2}$ families were produced in an isolated field in Clayton, NC, in 2002. Plants of all families were detasseled and used as female parents. Between every four rows of female lines, two rows of the F_1 hybrid tester FR615 × FR697 were planted on multiple dates to ensure pollination. FR615 and FR697 are non–stiff stalk type lines unrelated to FR1064 and GE440. Hybrid topcross seed was harvested from female rows.

Field Evaluation

In 2003 and 2004, an experiment was planted in four locations per year in North Carolina: the Central Crops Research Station at Clayton, the Tidewater Research Station at Plymouth, the Peanut Belt Research Station at Lewiston, and the Sand Hills Research Station at Jackson Springs. Soils at the experiment sites are classified as Marlboro loamy sand (fine, kaolinitic, thermic Typic Paleudult) at Clayton, Portsmouth fine sandy loam (fine-loamy over sandy or sandy-skeletal, mixed, thermic, Typic Umbraquult) at Plymouth, Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudult) at Lewiston, and Candor sand (sandy, siliceous, thermic Grossarenic Kandiudult) at Jackson Springs. Experimental entries included the 213 BC_1–derived family topcrosses, the two parental line topcrosses [FR1064 × (FR615 × FR697) and GE440 × (FR615 × FR697)], and seven check cultivars (B73 × Mo17; Pioneer brand hybrids 31G66, 31G98, 32R25, and 33M54; and Dekalb brand hybrids DK697 and DK743). Because topcross seed was limited for two BC_1–derived family topcrosses, those entries were replaced by a check hybrid (Pioneer brand hybrid 32K61) in some plots.

The treatment design was a sets design, wherein each $BC_1F_{1:2}$ family topcross hybrid was randomly assigned to one of three sets. Each set contained 71 $BC_1F_{1:2}$ topcrosses, the two parent line topcross hybrids, and seven check hybrids. The experimental design was a replication-within-sets design, and each set was arranged as an 8 by 10 α-lattice with two replications at each location. A sets design was used because the size of fields at some research stations prohibited use of a single large incomplete block design. Sets were planted in adjacent field blocks at each location. Experimental units were two-row plots 3.05 m in length, separated by 1.22-m alleys. Interrow spacing was 0.914 m in Lewiston and 0.9652 m in Clayton, Plymouth, and Jackson Springs. Plots were overseeded and thinned to target population densities of 44 plants per plot (62,288 plants ha^{-1} in Clayton, Plymouth, and Jackson Springs, or 65,750 plants ha^{-1} in Lewiston), or less in some environments because of poor germination due to cold

weather. For Clayton, the target stands for 2003 and 2004 were 31 and 44 plants per plot, respectively. For Plymouth, the target stands for 2003 and 2004 were 38 and 44 plants per plot, respectively. For Lewiston, the target stands for 2003 and 2004 were 28 and 44 plants per plot, respectively. For Jackson Spring, the target stands for 2003 and 2004 were 28 and 44 plants per plot, respectively. Stand counts were then taken approximately 4 wk after planting and plots were thinned manually to the target population density, if needed.

When all plants had reached physiological maturity, ear and plant heights were measured on six plants per plot. Plant height was measured from the ground to the terminal node; ear height was measured from the ground to the node of the topmost ear. Counts were taken of root-lodged plants (leaning greater than 30% from vertical with intact stalks) and stalk-lodged plants (broken below the ear or plants with dropped ears). Plots were harvested mechanically, and grain yield and grain moisture were measured.

Genotyping and Linkage Map Construction

The procedure for the development of the linkage map is detailed in Robertson-Hoyt et al. (2006). Briefly, young leaves from eight plants per family and parent line were sampled for DNA extraction. DNA extractions were performed using the DNeasy Plant Mini Kit system (Qiagen, Inc., Valencia, CA). Simple sequence repeat (SSR) primers were selected for screening in both populations using consensus map information from the Maize Genetics and Genomics Database (www.maizegdb.org) to reference locations of marker loci. Parental lines were screened with SSR primers to identify polymorphisms (Senior et al., 1998). Polymerase chain reaction (PCR) products were separated by electrophoresis in 4% SFR agarose gels (Amresco, Solon, OH) and visualized by staining gels with 0.05% ethidium bromide and exposing them to ultraviolet light.

Locus orders and recombination frequencies were estimated using multipoint mapping in MapmakerEXP (Whitehead Institute, Cambridge, MA). Recombination frequencies were then transformed to centimorgans (cM) using Haldane's mapping function in MapmakerEXP. The resulting map consisted of 105 loci distributed across the 10 chromosomes and was consistent with consensus genetic maps of maize (www.maizegdb.org). Average intermarker distance was 20.7 cM and the largest gap between markers was 57.6 cM (Robertson-Hoyt et al., 2006).

Statistical Analyses

Estimation of Family Means

Yields were adjusted to 155 g kg^{-1} grain moisture. Percent stand was computed as the plot stand count divided by the target stand for the given environment. Percent lodging was computed for each plot as number of root- or stalk-lodged plants divided by plot stand. Traditional analyses of variance and spatial analyses were performed on the data for each set within each environment separately to estimate genotypic least square means for each trait within each set and environment (Brownie et al., 1993). Models with up to fourth-order polynomial effects of row and columns in the field layout were tested. Trend effects were maintained if significant in the model at $P < 0.01$ (Brownie et al., 1993). The following models were compared using PROC MIXED in SAS version 8.2 (Littell et al., 1996; SAS Institute, 1999): a model including complete and incomplete block effects, a model with significant row and column trend effects, a model with correlated errors, and a model with both significant trend effects and correlated errors (Brownie et al., 1993). Percent stand was included as a covariate if significant at $P = 0.01$. For each set within each environment, the model that minimized Akaike's Information Criterion (Lynch and Walsh, 1997) was chosen. Entry least square means from each set within each environment were then calculated using the most appropriate statistical model. These least square means were then used as the basis for a combined analysis across all experiments and environments. Before conducting a combined analysis across environments, we checked for heteroscedasticity for grain yield using Bartlett's test (Milliken and Johnson, 1992). The combined analysis across environments and sets was based on the model:

$$Y_{ijk} = \mu + E_i + S_j + SE_{ij} + G_k + GE_{ik} + \varepsilon_{ijk}$$

where μ = overall mean; E_i = effect of environment i; S = effect of set j; SE_{ij} = interaction of environment i and set j; G_k = effect of genotype k; GE_{ik} = effect of interaction between environment i and genotype k; and ε_{ijk} = residual error. In this analysis, the residual variation is based on the means of check cultivars repeated across different sets (the interaction between set and repeated check genotypes). For the purposes of comparing the experimental entries to the parental lines (in hybrid combination) and hybrid checks, genotypes were considered to be fixed effects (Holland et al., 2003) and all other effects were considered to be random effects. This mixed model was analyzed using PROC MIXED in SAS and the least-square means of parents and hybrid checks adjusted for set effects were obtained and the average standard error of genotype mean comparisons was estimated for each trait. As part of this analysis, contrasts were used to test the null hypothesis that the 10 BC_1–derived families with lowest mean fumonisin concentration as lines per se in the experiment by Robertson et al. (2006) were not significantly different for agronomic traits in topcrosses than the 10 BC_1–derived families with highest mean fumonisin concentration in the same study. This two-step analysis based on combining results of individual environment analyses is not maximally efficient relative to a single combined analysis, but the use of different spatial analysis models in different environments prevented simple application of a single mixed model for this data set.

For the purposes of estimating genetic variance components, parental topcrosses and hybrid checks were dropped from the data set and genotypes were considered random effects. Heritabilities for each trait on an entry mean basis were estimated from the univariate mixed model analyses using PROC MIXED of SAS (Holland et al., 2003). Approximate standard errors for heritability estimates were estimated with the delta method as described by Holland et al. (2003). PROC CORR of SAS (SAS Institute, 1999) was used to estimate the correlations between the genotypic means of the different traits measured in the hybrid trials with the means of fumonisin contamination and Fusarium ear rot measured in an inbred study (Robertson et al., 2006). This was performed to approximate a genotypic correlation between traits, because ear rot and fumonisin were measured mostly in different environments than the topcross agronomic traits. There is a small bias due to genotype-by-environment covariances in this estimator because two of the

eight yield-trial environments coincided with two of the four disease-evaluation environments (Casler, 1982).

QTL Detection and Estimation

Quantitative trait loci were identified using the procedure described in Robertson-Hoyt et al. (2006) based on the genotypic least square means across environments. Briefly, composite interval mapping (CIM) was implemented in Windows QTL Cartographer 2.5 only to provide initial models for further analysis by the multiple interval mapping (MIM) method. QTL peaks from the CIM analysis, with a LOD threshold value of 2.0 and minimum 5 cM between QTL, were used as the initial models for MIM in Windows QTL Cartographer 2.5. Models were then created and tested in an iterative, stepwise fashion, searching for new QTL to add to the model, and testing the significance of the QTL after each search cycle. New models were accepted if they decreased the Bayesian Information Criterion (BIC) (Piepho and Gauch, 2001). After no additional QTL could be added to the model according to the BIC, each pair of QTL in the model was then tested for epistatic interactions. Epistatic interactions were kept in the model if they decreased the BIC. While developing the model using multiple interval mapping, we were aware that overfitting the model was possible. Bogdan et al. (2004) proposed a modification of the BIC with improved model selection properties, but not all statistics required to calculate this modified BIC are available from QTL Cartographer. Therefore, to avoid model overfitting, we checked if the proportion of total variation due to QTL exceeded the trait heritability. If this occurred, the QTL with smallest effect was dropped from the model, and the model was retested. If the model still explained a higher proportion of the phenotypic variance than the heritable proportion, the QTL with smallest effect in this reduced model was dropped. This process was continued until we obtained the best model according to the BIC that did not explain a greater proportion of the variation than the heritability of the trait.

When no additional QTL main effects could be added to the model, the model with minimum BIC was chosen and QTL effects were simultaneously estimated using the "summary" option of QTL Cartographer. Genetic variability explained by QTL for each trait was calculated as the total phenotypic variation explained by QTL divided by the entry mean heritability of the trait. When comparing QTL for two or more traits, QTL positions were considered to be overlapping when they were within 20 cM (Visscher et al., 1996; Melchinger et al., 1998; Cardinal and Lee, 2005).

To test the null hypothesis that the proportion of loci at which BC_1–derived lines possessed GE440 alleles had no effect on topcross agronomic performance, we estimated correlations between proportion of segregating loci in the BC_1–derived lines and topcross trait means of each line.

RESULTS

We tested for heteroscedasticity for yield using Bartlett's test (Milliken and Johnson, 1992) and found no evidence for differences in residual error variances across environments ($P > 0.90$). Therefore, we conducted a combined analysis across environments based on the adjusted least square means from within each environment. We treated genotypes as fixed effects for the purposes of estimating least square means and making comparisons to check cultivars, and as random effects for the purpose of estimating variance and covariance components. Piepho et al. (2006) have recently proposed a more efficient analysis of treatment structures containing mixtures of fixed and random entries. We compared genotype least square means from our original analysis and best linear unbiased predictors from an analysis based on the model of Piepho et al. (2006) (but not incorporating within-environment spatial trend adjustments) for grain yield. Even without appropriately accounting for spatial trends in the latter analysis, the least square means and best linear unbiased predictors were highly correlated ($r = 0.92$, $P < 0.0001$). Therefore, we considered the least square means appropriate estimators for the purposes of comparing genotypes and detecting QTL.

$BC_1F_{1:2}$ topcrosses differed significantly ($P < 0.0001$) for all traits except for root lodging, for which entries did not significantly differ. Because root lodging values were not significantly different, root lodging was not included in any further analyses. The hybrids created from the two parental lines did not differ significantly for days to silking or anthesis. However, the GE440 topcross hybrid had significantly higher values than the FR1064 topcross hybrid for grain yield, grain moisture, plant height, ear height, percent stalk lodging, and percent target stand.

We found evidence of transgressive segregation for three of the traits measured. No family hybrids had significantly lower mean yield than the FR1064 topcross, but one family hybrid had significantly higher mean yield than the GE440 topcross across environments (Table 1). Two family hybrids had significantly lower mean grain moisture than the FR1064 topcross, but no family hybrid had significantly higher mean grain moisture than the GE440 topcross. Two family hybrids mean silking dates were significantly earlier than the FR1064 topcross, but no family hybrid mean silking date was later than the GE440 topcross. No transgressive segregation was detected for lodging, plant height, ear height, anthesis date, or target stand (Table 1).

Comparisons of Highest and Lowest Fumonisin Contaminated Families

The relationship between disease resistance and topcross agronomic performance was tested by comparing the mean topcross values for each trait of the 10 families with lowest mean fumonisin concentration to the means of the 10 families with highest mean fumonisin concentration. The fumonisin data were taken from the study of the families as lines per se by Robertson et al. (2006) These two groups were significantly different for three traits in topcross evaluations: stalk lodging ($P = 0.0168$), grain moisture ($P < 0.0001$), and percent stand ($P < 0.0001$). The family hybrids created from the 10 families with lowest fumonisin concentration averaged 6%

Table 1. Mean fumonisin concentrations and Fusarium ear rot means measured by Robertson et al. (2006) in $BC_1F_{1:2}$ lines in four North Carolina environments and means of topcross agronomic traits measured in eight North Carolina environments for parental lines and each of highest 10 and lowest 10 fumonisin contaminated families.

Family	Traits measured in $BC_1F_{1:2}$ lines per se		Traits measured in topcrosses						
	Inbred fumonisin	Inbred rot	Grain yield	Grain moisture	Stalk lodging	Plant height	Ear height	Silk date	Anthesis date
	μg g^{-1}	%	Mg ha^{-1}	g kg^{-1}	%	cm		DAP†	
Families with lowest fumonisin concentration									
397-8	0.4 (1.5)‡	<1	7.54	162.0	8	214	95	60	59
399-3	1.2 (3.3)	3	8.82	159.1	7	213	92	60	59
400-9	1.2 (3.3)	17	8.14	159.0	9	209	92	60	59
397-10	1.2 (3.3)	7	7.73	154.3	9	210	94	61	61
412-13	1.3 (3.7)	21	9.17	154.9	6	216	85	–	–
396-14	1.3 (3.7)	11	7.72	154.3	5	214	90	60	59
402-3	1.4 (4.1)	8	8.00	158.0	6	213	98	60	59
399-13	1.4 (4.1)	2	7.80	159.0	6	211	94	60	60
400-3	1.4 (4.1)	7	7.52	156.4	4	209	88	60	58
399-1	1.5 (4.5)	6	7.98	155.4	4	213	92	60	59
Mean (Lowest 10)	1.2 (3.3)	9	8.04	157.2	6	212	92	60	59
Families with highest fumonisin concentration									
402-10	3.4 (30.0)	25	7.96	150.2	4	210	92	61	59
405-1	3.5 (33.1)	23	7.72	151.1	6	218	92	59	58
402-11	3.5 (33.1)	30	8.01	156.8	4	215	90	61	60
401-6	3.5 (33.1)	27	8.09	151.5	4	210	87	60	60
397-13	3.5 (33.1)	22	7.49	158.4	7	214	99	61	60
403-11	3.5 (33.1)	19	7.61	160.8	4	215	92	60	60
399-7	3.6 (36.6)	23	7.92	158.0	4	222	96	61	60
410-4	3.6 (36.6)	36	7.71	143.4	4	207	87	60	59
413-5	3.7 (40.4)	26	7.60	145.8	5	207	84	57	57
413-7	4.2 (66.7)	32	8.65	151.4	4	213	91	60	59
Mean (highest 10)	3.6 (36.6)	26	7.88	152.7	5	213	91	60	59
Lowest 10 mean – highest 10 mean§	–2.4 (–33.3)***	–17***	NS	4.5***	1*	NS	NS	NS	NS
$BC_1F_{1:2}$ family overall mean	2.5 (12.2)	18	7.96	154.4	5	212	92	60	59
Range of families	0.4–4.2 (1.5– 66.7)	0–36	6.81–9.87	143.4–163.7	2–10	200–225	81–101	57–62	57–61
GE440 × Tester	0.8 (2.2)	11	8.33	166.3	17	228	114	61	60
FR1064 × Tester	3.3 (27.1)	22	7.07	151.4	4	205	84	60	59
Commercial check mean	NA¶	NA	9.74	163.7	5	220	94	62	61
LSD (0.05)#	0.09 (NA)	13	0.78	0.6	4	7	5	2	2

*Significant at $P < 0.05$.

***Significant at $P < 0.001$.

†DAP, days after planting.

‡Values outside of parentheses are natural log transformed means. Values in parentheses represent means back-transformed to approximate their original value of μg g^{-1} (Robertson et al., 2006).

§Differences between the hybrids created from the highest 10 and lowest 10 fumonisin-contaminated families were estimated to test the null hypothesis that the ten BC_1–derived families with lowest mean fumonisin content as lines per se in the experiment by Robertson et al. (2006) were not significantly different for agronomic traits in topcrosses than the 10 BC_1–derived families with highest fumonisin content in the same study.

¶NA, not applicable.

#The LSD shown is appropriate for comparing individual hybrids.

stalk-lodged plants per plot, while the 10 families with highest fumonisin content averaged 5% (Table 1). For grain moisture, the family hybrids created from the 10 families with lowest fumonisin content averaged 157.2 g kg^{-1} while the 10 families with highest fumonisin content averaged 152.7 g kg^{-1} (Table 1). For percent stand, the family hybrids created from the 10 families with the lowest fumonisin content averaged 97% target stand while the 10 families with the highest fumonisin content averaged 88% target stand.

Heritabilities

Heritabilities on an entry mean basis for traits measured ranged from moderately low to high: grain yield (h^2 = 0.54, SE = 0.05), grain moisture (h^2 = 0.65, SE = 0.04), stalk lodging (h^2 = 0.30, SE = 0.07), plant height (h^2 = 0.80, SE = 0.02), ear height (h^2 = 0.84, SE = 0.02), days to silking (h^2 = 0.37, SE = 0.09), days to anthesis (h^2 = 0.44, SE = 0.08), and percent target stand (h^2 = 0.84, SE = 0.02).

Correlations

Many of the correlations between Fusarium ear rot or fumonisin concentration of $BC_1F_{1:2}$ families per se and agronomic traits measured in the hybrid study were significant. Significant correlations ranged from $r = -0.30$ ($P < 0.0001$, between Fusarium ear rot and moisture content) to $r = 0.29$ ($P < 0.0001$ between Fusarium ear rot and yield; Table 2). The correlation between the proportion of 105 marker loci in $BC_1F_{1:2}$ lines that had GE440 alleles and topcross yield was positive ($r = 0.17$, $P = 0.016$). This indicates that, in general, having more GE440 alleles improved topcross yield, although the correlation was low. The proportion of loci in $BC_1F_{1:2}$ lines that had GE440 alleles was also correlated positively with topcross grain moisture ($r = 0.34$, $P < 0.0001$), plant height ($r = 0.30$, $P < 0.0001$), and ear height ($r = 0.34$, $P < 0.0001$), indicating that, in general, the GE440 genetic background is associated with increased grain moisture content as well as increased plant and ear heights.

QTL Identified

We identified seven QTL for grain yield, five for grain moisture, eight for plant height, six for ear height, and three for silk date and four QTL with one epistatic interaction for anthesis date (Table 3). No stalk lodging QTL were identified. Considering all agronomic traits, QTL were detected on all 10 chromosomes. Combined QTL models for each trait accounted for 14 to 60% of the phenotypic variation and for 39 to 72% of the genotypic variation (Table 3).

For all traits, except grain yield and anthesis date, most of the favorable alleles were derived from FR1064. For grain yield, the GE440 allele was favorable at five of seven QTL, but the FR1064 allele was favorable at the two yield QTL with largest effects (which explained 7.8 and 9.2% of the variation among family means, Table 3). The GE440 allele was associated with earlier anthesis at three of four silk date QTL (Table 3).

Comparison of Disease Resistance QTL and Agronomic Performance QTL

Fusarium ear rot was positively correlated with topcross yield (Table 2), but no Fusarium ear rot QTL (reported in Robertson-Hoyt et al., 2006) mapped to the same positions as yield QTL. In contrast, despite the lack of significant correlation between fumonisin content and topcross yield, two fumonisin contamination QTL mapped to similar positions as yield QTL. In the region of 95.00 to 109.8 cM on chromosome 1, the fumonisin QTL (which accounted for 10% of the phenotypic variation) overlapped the yield QTL with the largest effect. On chromosome 7, in the region between 24 and 30 cM, a fumonisin-contamination QTL (which accounted for 7% of the phenotypic variation) overlapped a yield QTL. The GE440 allele contributed to reduced fumonisin content at all QTL, but the GE440 allele was associated with reduced yield at the overlapping yield QTL on chromosome 1, whereas the GE440 allele was associated with increased yield at the overlapping QTL on chromosome 7. The fumonisin QTL on chromosome 1 is also overlapping with a plant height QTL (FR1064 beneficial allele) and an ear height QTL (FR1064 beneficial allele) (Table 3; Robertson-Hoyt et al., 2006).

Fumonisin contamination and Fusarium ear rot were both positively correlated with stalk lodging and negatively correlated with grain moisture (Table 2). However, there were no lodging QTL identified, and Fusarium ear rot and fumonisin contamination QTL did not overlap with grain moisture QTL (Table 3; Robertson-Hoyt et al., 2006).

Fusarium ear rot and plant height were positively correlated, and one region on chromosome 5 (83–100 cM) contained QTL for both traits. The favorable alleles for both traits (shorter plants and lower ear rot) in this region are from GE440. There are three QTL overlapping between fumonisin concentration and plant height, even though these two traits were not significantly correlated (chromosome 1, 95–104 cM; chromosome 4, 135–145 cM; and chromosome 5, 83–97 cM).

DISCUSSION

Unexpectedly, the GE440 topcross hybrid had significantly higher yield than the FR1064 topcross hybrid, and no BC_1 family hybrid yielded significantly less than FR1064. This was surprising because the GE440 inbred is tall with spindly, weak stalks and has very small, poorly filled ears, whereas FR1064 is an improved B73 line that

Table 2. Correlations between means for fumonisin contamination or Fusarium ear rot measured in an inbred study (Robertson et al., 2006) with agronomic traits measured in topcross hybrids.

	Grain yield	Grain moisture	Stalk lodging	Plant height	Ear height	Silk date	Anthesis date	Target stand
Fumonisin concentration	NS†	$r = -0.29$ $P < 0.0001$	$r = 0.17$ $P = 0.0132$	NS	NS	NS	NS	$r = -0.20$ $P = 0.0034$
Fusarium ear rot	$r = 0.29$ $P < 0.0001$	$r = -0.30$ $P < 0.0001$	$r = 0.18$ $P = 0.0078$	$r = 0.15$ $P = 0.0285$	NS	NS	NS	$r = -0.25$ $P = 0.0002$

†NS, not significant at $P = 0.05$.

Table 3. Quantitative trait loci (QTL) identified with multiple interval mapping for traits measured in the hybrid trials.

Trait†	Beneficial allele	Chromosome	QTL position	Bin	Left marker	Right marker	Effect‡	% phenotypic variation explained
Yield	FR1064	1	109.08	1.04	bnlg1811	bnlg1884	0.23	9.2
Yield	GE440	4	106.54	4.07	umc1329	dupssr34	–0.16	5.1
Yield	GE440	5	220.15	5.09	bnlg1118	umc1153	–0.18	5.8
Yield	GE440	7	30.18	7.02	umc2098	umc1134	–0.13	2.7
Yield	GE440	8	82.18	8.05	bnlg666	bnlg1031	–0.14	3.5
Yield	GE440	9	245.67	9.07	bnlg1375	umc1982	–0.15	3.6
Yield	FR1064	10	37.45	10.02	mmc0501	umc1785	0.19	7.8
							Total§	37.7
MOIS	GE440	1	0.01	1.01	phi056	bnlg1124	0.7	0.9
MOIS	GE440	1	192.58	1.09	umc1085	umc2047	1.8	5.6
MOIS	FR1064	2	106.17	2.04	bnlg1175	bnlg1036	–2.1	7.7
MOIS	FR1064	3	80.14	3.04	bnlg2136	bnlg1452	–1.2	3.1
MOIS	FR1064	6	111.29	6.05	bnlg1702	umc2375	–2.9	14.0
							Total	31.3
PHT	FR1064	1	103.99	1.04	phi001	bnlg1811	–2.7	6.2
PHT	FR1064	3	91.06	3.04	bnlg1452	phi053	–1.9	3.4
PHT	GE440	4	134.75	4.08	bnlg2244	umc1086	2.9	8.6
PHT	GE440	5	83.32	5.03	umc1355	umc2111	0.9	0.4
PHT	FR1064	5	215.15	5.09	bnlg1118	umc1153	–3.8	11.7
PHT	FR1064	6	0.01	6.02	umc1257	umc1014	–2.5	6.5
PHT	FR1064	8	54.93	8.05	bnlg1812	bnlg666	–2.8	7.5
PHT	FR1064	9	161.16	9.05	bnlg1270	bnlg1375	–3.6	12.9
							Total	57.2
EAR	FR1064	1	99.99	1.04	phi001	bnlg1811	–2.1	4.6
EAR	FR1064	3	110.49	3.06	phi053	bnlg1449	–4.1	22.7
EAR	GE440	4	139.75	4.08	bnlg2244	umc1086	2.1	7.4
EAR	FR1064	6	0.01	6.02	umc1257	umc1014	–1.8	5.1
EAR	FR1064	8	54.93	8.05	bnlg1812	bnlg666	–2.6	8.5
EAR	FR1064	9	173.16	9.07	bnlg1270	bnlg1375	–2.8	11.5
							Total	59.8
SILK	GE440	4	116.95	4.07	dupssr34	bnlg2244	0.3	5.1
SILK	FR1064	8	61.18	8.05	bnlg1812	bnlg666	–0.3	2.7
SILK	FR1064	9	150.16	9.05	bnlg1270	bnlg1375	–0.4	6.6
							Total	14.4
ANTH	GE440	2	209.48	2.09	bnlg1520	umc2214	0.3	4.4
ANTH	GE440	4	63.70	4.05	umc2280	umc2061	0.2	1.3
ANTH	GE440	7	45.18	7.03	umc2098	umc1134	0.2	2.3
ANTH	FR1064	9	245.67	9.07	bnlg1375	umc1982	–0.3	4.1
ANTH		4*7¶					0.6	5.0
							Total	17.1

†Yield effect is in Mg ha^{-1}, grain moisture (MOIS) is in g kg^{-1}, plant height (PHT) and ear height (EHT) are in cm, and effects of average midsilk (SILK) and anthesis (ANTH) date are in d.

‡QTL effect estimated as the difference between homozygous FR1064 genotypes and segregating GE440/FR1064 genotypes.

§Total variation associated with all of the QTL in the model fitted simultaneously.

¶Epistatic interaction is presented using the chromosome to designate which QTL is involved in the interaction.

was widely used for commercial hybrid production in the recent past. The superiority of GE440 was also observed in the positive correlation between the proportion of loci in $BC_1F_{1:2}$ lines that contained GE440 alleles and topcross grain yield, although the correlation was low (r = 0.17). Finally, at five of the seven yield QTL identified, the GE440 allele was associated with increased yield. These results suggest that GE440 may have a more suitable background in hybrid combination with tester FR615 × FR697 for increasing yield under growing conditions in North Carolina. The greater topcross yield of GE440 may be due in part to greater heterosis due to greater genetic distance

from the tester. It may also be due to better local adaptation of GE440 (developed in Georgia) to North Carolina environments than FR1064 (developed by Illinois Foundation Seeds, Inc. mainly from a midwestern USA adapted line, B73). Finally, under conditions conducive to Fusarium ear rot, the resistance alleles in GE440 and its progeny may have contributed to greater yields directly by preventing loss due to ear rot.

The FR1064 topcross hybrid's poor yield was also likely associated with its poor stand (61% of target stand compared with 98% for GE440 topcross) (Table 1), despite our attempt to adjust yield for differences in stand by using stand as a covariate in the analysis. A similar significant difference was observed between the high fumonisin progeny mean stands (88%) and the low fumonisin group mean stands (97%), which also had higher and lower mean ear rot, respectively (Robertson et al., 2006). The topcross seeds were produced using GE440, FR1064, or the BC_1–derived family as the female parent in an environment prone to Fusarium ear rot. Therefore, it is likely that the FR1064 topcross and the more susceptible family topcrosses had higher proportions of seed that was damaged by *Fusarium* spp., leading to lower germination and lower stands. However, although there was a difference between the yield of the two parental topcross hybrids, there was no significant yield difference between the two BC_1–derived family groups (Table 1). This suggests that using stand as a covariate in our analyses corrected for differences in stand within part of the range of variability, but perhaps was not adequate to adjust for the extremely low stands of the FR1064 topcross. These results further suggest that backcrossing Fusarium ear rot resistance alleles from GE440 into the FR1064 background could broaden that range of adaptation of FR1064 to include environments that are conducive to Fusarium ear rot, such as North Carolina (Shelby et al., 1994).

Based on the percentage of genotypic variation explained by QTL (Table 3), we were able to identify QTL associated with the majority of genetic variation for yield, plant height, and ear height (in each case, the combined effects of mapped QTL explained greater than 70% of the genotypic variation). Many QTL influencing moisture were identified. However, most of the QTL identified for moisture had small effects, suggesting that there may be many very small effect QTL that were not identified. For silk date and anthesis date, however, only a small number of QTL were identified (for silk and anthesis date, QTL explained only 39% of the genotypic variation) and for lodging no QTL were identified. For silk and anthesis date, the low number of QTL identified could be due to low power of detection, because only two environments were used to collect data on flowering time. Flowering time in hybrids, however, was not correlated with resistance to either fumonisin or ear rot, which confirms the results measured in lines per se by Robertson et al. (2006) and demonstrates that resistance is not due to disease escape due to flowering-time effects. We were unable to map QTL with effects on mean lodging ratings because a large proportion of the variation in stalk lodging was due to genotype by environment interaction.

Grain moisture was negatively correlated with Fusarium ear rot and fumonisin contamination. Bush et al. (2004) reported that early harvest (to force dry down as fast as possible) is a good control strategy for reducing fumonisin contamination, by escaping the disease pressure that would lead to high levels of fumonisin. The correlation we observed in this study between lines with low mean fumonisin and topcrosses with higher moisture concentration is likely due to linkage between high moisture alleles and disease resistance alleles in GE440. This illustrates the commonly observed phenomenon that introgression of unadapted germplasm is often accompanied by unexpected and possibly unwanted phenotype change along with favorable change (Goodman, 1985).

The results of the parental line comparisons, correlation analysis, and QTL analysis were not always similar. For example, there was a positive, but small ($r = 0.15$), correlation between Fusarium ear rot and plant height. This was surprising because GE440 had significantly lower ear rot than FR1064 and its topcross was significantly taller than the FR1064 topcross. However, at the one QTL region that affected both Fusarium ear rot and plant height, the GE440 allele was associated with shorter plants. Therefore, this QTL region contributes to the positive correlation between these two traits. Another example was that the trait pair that had the greatest number (three) of overlapping QTL (fumonisin content and plant height) was not significantly correlated. Finally, two overlapping QTL for fumonisin concentration and grain yield were discovered, despite the lack of correlation between these two traits. Careful consideration of these results, however, reveals that different QTL often contributed in opposite directions to the total genetic covariance between the two traits. For example, of the three QTL regions that affected both fumonisin concentration and plant height, the GE440 allele of the two most important QTL had nearly identical effect magnitudes but opposite signs on plant height (−2.7 cm on chromosome 1 and +2.8 cm on chromosome 4) (Table 3). Similarly, for grain yield and fumonisin concentration, the overlapping QTL region on chromosome 1 would have contributed a positive genetic covariance, whereas the region on chromosome 7 would have contributed a negative genetic covariance. Because the contributions of these unlinked gene regions would be summed in their respective total genetic covariances, they would have partly canceled each other out in both cases.

In general, marker-assisted selection could prove useful in helping to break linkages between resistance QTL and other important agronomic traits if backcrossing resistance QTL is the breeding goal. However, the QTL identified in this study explain such a small percentage of the variation

that marker-assisted selection for improving topcross agronomic traits cannot be recommended. In summary, the correlation results suggest that if ear rot resistance alleles were backcrossed into FR1064, yield, stalk lodging, and plant height would decrease, while grain moisture content would increase. If fumonisin contamination resistance alleles were backcrossed into FR1064, stalk lodging would decrease, while grain moisture content would increase. In both cases, a backcross breeding program to introgress resistance alleles from GE440 into FR1064 appears promising because the results of this study predict only minor negative impacts on testcross agronomic performance of such introgression.

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